抗坏血酸的代谢和调控—以模式植物和园艺植物为例

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摘要 抗坏血酸在植物的生长发育和抗逆过程中具有重要作用。主要概述了抗坏血酸在模式植物和园艺植物中的代谢途径，并且从转录水平、翻译水平和遗传转化等几个方面综述了多个不同基因对抗坏血酸水平的调控机制。以期为深入研究抗坏血酸在植物（尤其是园艺植物）中的代谢调控机制提供参考。

关键词 抗坏血酸；代谢；调控；模式植物；园艺植物

中图分类号 S601

1 前言

抗坏血酸（ascorbic acid，AsA）又名维生素C，为五碳糖的衍生物，在植物中广泛存在[1]。AsA在植物体内功能多样，参与细胞壁合成、细胞分裂与生长、光合作用、衰老和激素合成等生长发育代谢活动，在抵御脱水[2,3]、强光[4]、盐[5]和臭氧[6]等各类逆境胁迫的抗氧化保护方面亦具有重要的功能。AsA与人类健康关系密切，在癌症、心血管病、衰老及白内障等与氧胁迫相关疾病的防御过程中发挥重要作用[7,8]。因为人类缺乏自我合成AsA的能力，所以日常饮食中的果实和蔬菜是人类摄取AsA的重要来源。因此关于植物（特别是园艺植物）AsA的研究一直是植物学研究的热点之一。

近些年来，世界各国的多位学者针对植物AsA代谢机理展开了大量的研究。研究认为，植物AsA代谢具有两个显著特点：一是不同植物种类、品种和部位之间AsA含量差异明显[9]。比如猕猴桃[10]和金虎尾[11]的果实AsA含量能达到800mg/100g FW，而‘嘎啦’苹果果实的AsA含量只有5mg/100g FW[12]。野生种潘那利番茄（*Solanum pennellii*）比栽培番茄（*Solanum lycopersicum*）的AsA含量高5倍[13]。苹果叶片中的AsA含量也比果实中的高近100倍[12]。二是AsA含量受外界环境条件影响显著。光照[14]、温度[15]、相对湿度[9]和污染物质[16]等均能影响AsA的积累。以上这些特点表明在植物体内有着复杂的AsA代谢调控机制。鉴于AsA在植物体内的代谢途径，特别是合成途径和循环再生途径已经比较清楚，因此近年来关于植物AsA的研究重心已经转移至代谢调控机制方面。本文就近年来植物AsA代谢调控机制方面的研究进行综述。

2 AsA 在植物体内的代谢

AsA在植物体内的代谢包括合成（图1）、再生（图2）、运输（图3）和降解（图4）等几个方面。

目前的研究表明，植物可以通过四条途径生成AsA，分别是：L-半乳糖途径、L-古洛糖途径、D-半乳糖醛酸途径和肌醇途径（图1）。L-半乳糖途径是目前公认的大多数植物体内AsA合成的主要途径，也称为Smirnoff-Wheeler途径[17]。这一途径中D-葡萄糖首先经一系列磷酸化生成D-甘露糖-1-P；D-甘露糖-1-P再经焦磷酸化、表异构化和磷酸化等作用生成L-半乳糖；L-半乳糖最后经脱氢氧化和进一步脱氢生成AsA。这个途径中涉及的所有基因（酶）

收稿日期：　　　　　　修回日期：

基金项目：四川省国际猕猴桃生物技术育种平台项目（2016NZ0105）和四川省科技厅应用基础研究项目（2017JY0169；2017JY0054）

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目前均已被鉴定（图1）。

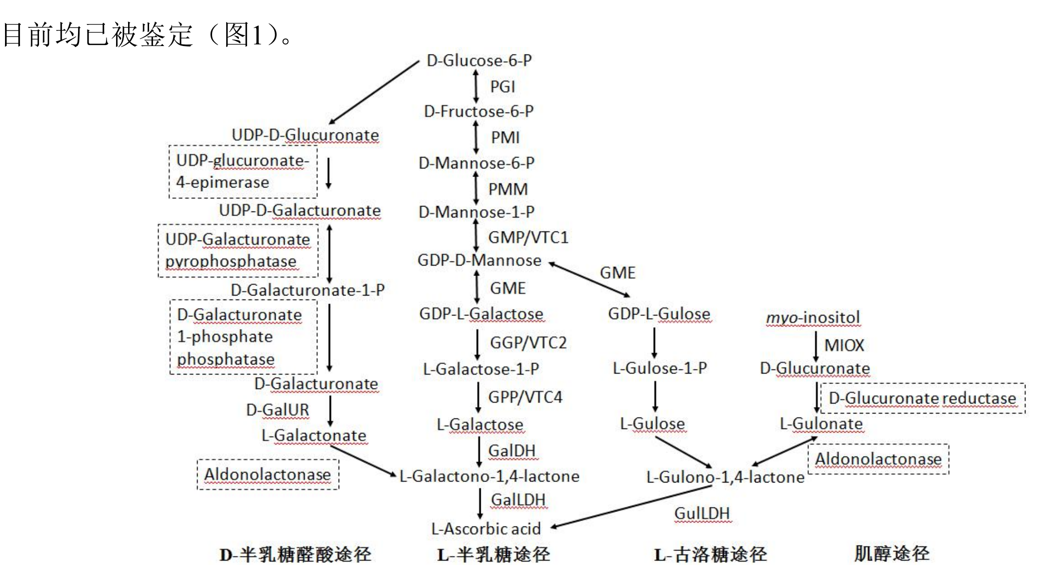


图 1 植物 AsA 生物合成途径

虚线方框的酶表示目前尚未得到鉴定

Fig.1 The pathways of AsA biosynthesis in plants

Enzymes in dotted box indicted that they were not identified until now

PGI:glucose-6-phosphate isomerase; PMI :mannose-6-phosphate isomerase; PMM :phosphomannomutase; GMP :GDP-mannose pyrophosphorylase; GME: GDP-mannose-3’,5’-epimerase; GGP: GDP-L-galactose phosphorylase; GPP :L-galactose-1-phosphate phosphatase; GalDH: L-galactose dehydrogenase; GalLDH :L-galactono-1,4-lactone dehydrogenase; MIOX: myo-inositol oxygenase; GalUR: D-galacturonate reductase; GulLDH: L-gulono-1,4-lactone dehydrogenase

D-半乳糖醛酸、D-葡萄糖醛酸、D-甘露糖和L-半乳糖都是细胞壁多糖（如果胶等）的主要合成物质，果胶等细胞壁多聚体降解形成甲基-D-半乳糖醛酸，再经去甲基化后形成D-半乳糖醛酸酯。D-半乳糖醛酸酯经D-半乳糖醛酸酯还原酶还原产生L-半乳糖醛酸，经醛内酯酶催化生成L-半乳糖酸-1,4-内酯合成AsA[18]。D-半乳糖醛酸酯途径在某些植物中是重要的AsA合成补充途径，如番茄和草莓[1,19]。研究发现GME除催化GDP-Man生成GDP-L-半乳糖外，也可催化GDP-D-甘露糖5′异构化生成GDP-L-古洛糖。古洛糖途径正是由GDP-L-古洛糖起始，经由L-古洛糖酸-1,4-内酯生成AsA。这一途径与动物中AsA合成的L-古洛糖途径较类似[20]。随后在拟南芥中又发现了依赖肌醇的合成途径。肌醇途径是指肌醇在肌醇加氧酶的作用下生成葡萄糖醛酸，然后在葡糖醛酸脱氢酶和醛糖酸酯化酶的作用下生成L-古洛糖-1，4内酯，进而参与到古洛糖途径生成AsA[21]。古洛糖途径、D-半乳糖醛酸酯途径和肌醇途径是一些植物中AsA生物合成的重要补充途径，但这些途径中涉及的基因（酶）目前只有部分被鉴定[22]（图1）。

AsA在植物体内是通过抗坏血酸-谷胱甘肽循环（Ascorbate-Glutathlione，AsA-GSH Cycle）实现再生的。在非生物逆境胁迫下，AsA首先作为APX的电子供体，可以清除逆境条件下产生的H_2O_2，而同时自身被氧化为MDHA。一部分MDHA可在依赖NAD（P）H的MDHAR的作用下被还原为AsA，另一部分又可通过非酶歧化反应生成DHA，而DHA在DHAR催化和GSH参与下又被还原为AsA，使H_2O_2最终被清除。该反应产生的GSSG在NADPH的存在下又可被GR催化还原为GSH[23]（图2）。

由于AsA生物合成的最后一步酶GaILDH定位于线粒体内膜，所以AsA是在线粒体内膜中合成，而在叶绿体、质体及液泡等其它亚细胞区域内AsA含量也很高，这表明AsA在合成后进行了细胞内部的跨膜转运，而线粒体中AsA的运出可能是通过浓度梯度的简单扩散机制[24,25]。现已发现，细胞核可以通过核孔自由进出AsA，叶绿体的AsA转运是通过载体介导的，类囊体和液泡的AsA运输依赖于浓度和酸碱性梯度的简单扩散过程，但过氧化物酶体中的机制还不清楚[26,27]。不同于细胞内，质外体不存在AsA-GSH循环中的任何酶，导致质外体中

的AsA不能被循环利用，因此需要原生质体和质外体之间进行AsA和DHA的跨膜运输。这种跨细胞膜的运输分为依赖于电化学梯度的运输和蛋白运输载体介导[28,29]两类。除了跨膜载体外，细胞色素b（Cyt b）也能间接的引起AsA转移。Cyt b作为电子传递体偶联在细胞膜上，可利用胞质AsA 氧化所产生的电子，将质外体的MDHA还原[30]（图3），使胞外的AsA氧化还原状态处于平衡态。

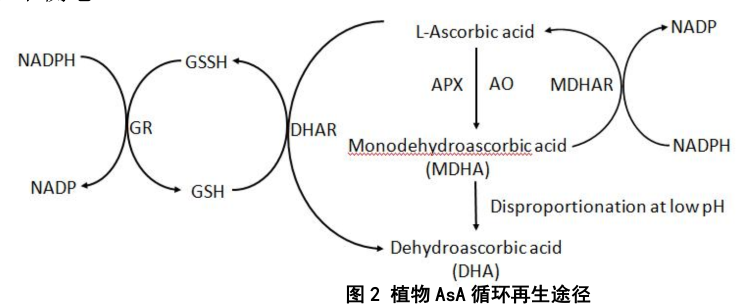


图 2 植物 AsA 循环再生途径

Fig.2 The recycling pathway of AsA in plant

APX: ascorbate peroxidase; MDHAR: monodehydroascorbate reductase ; DHAR: dehydroascorbate reductase; AO: ascorbate oxidase; GR: glutathione reductase

目前对于植物细胞原生质膜上是否存在专一的 AsA 或 DHA 运载体及其与其它运载蛋白的关系仍不清楚。目前只在拟南芥中发现 12 个编码膜蛋白的基因与碱基/抗坏血酸转运体（nucleobase/ascorbate transporter，NAT）同源。但拟南芥 NAT 双突变体和三缺失突变体都未见表型变化，表明 NAT 功能在植物中高度冗余。NAT 是否负责 AsA 的转运及其机制目前也不清楚[31]。

除胞内和胞间短距离运输外，AsA 是否还能通过韧皮部进行长距离运输，目前争议很大。很多研究发现在植物的组织中，AsA 的合成几乎是无处不在，包括源器官、输导组织和库器官[32,33,34]，这使 AsA 运输的必要性受到质疑，而且现在也没有发现 AsA 的韧皮部装载和卸载 。但另一些同位素示踪的研究却发现 AsA 可能会进行韧皮部的长距离运输[35,32]。

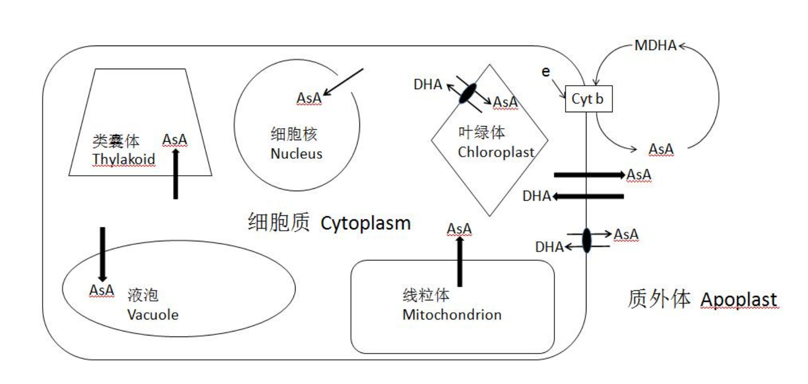


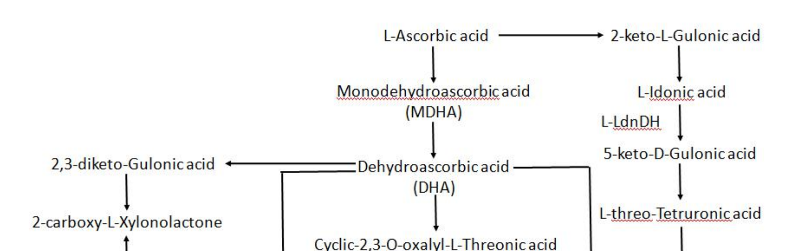
图 3 植物细胞中 AsA 的运输

：分别代表电化学浓度梯度扩散、自由扩散和载体介导运输；e：电子

Fig.3 The transportation of AsA in plant cells

：means gradient diffusion of electrochemical concentration, free diffusion and transportation via transportor；e：electron

AsA 和 DHA 在不能及时还原的情况下就开始发生降解。降解可以通过酶或非酶途径进行，酒石酸（盐）、草酸（盐）和苏糖酸（盐）是主要的降解产物。酒石酸的形成主要是因为 AsA 的碳链在 C4/C5 位置发生断裂，因此酒石酸的形成来源于抗坏血酸的 1-4 号位置的碳原子。这个降解途径主要发生在葡萄等葡萄科植物中，目前发现的中间产物有 2-酮-L-古洛糖酸、L-艾杜糖酸、L-苏式丁糖酮酸等，该途径中目前只有 L-艾杜糖脱氢酶（L-Idonate dehydrogenase）得到鉴定[36]。在葡萄等植物中酒石酸主要钙盐或磷酸盐的形式存在[37]（图 4）。



草酸和苏糖酸的形成主要是因为AsA的碳链在C2/C3位置发生断裂。一些研究表明，从DHA到草酸和苏糖酸的中间产物依次为环状草酰苏糖酸和草酰苏糖酸[38]，但另一些研究表明，在氧化条件较强时，环状草酰苏糖酸、草酰苏糖酸、草酸和苏糖酸是同时产生的[39]。这个降解途径发现于不同植物的质外体、各类细胞器和体外试验。体外试验发现这个途径可以不依赖酶的催化进行，但有酶催化时反应更加迅速[38]。另一方面，在较弱的氧化条件下，DHA还可以被水解为2，3-二酮古洛糖酸，在异构化等作用下经2-羧基-L-苏式戊糖酸后形成L-苏糖酸[40]。在天竺葵属植物中还发现L-苏糖酸可以进一步生成酒石酸[41]。还有研究发现通过以上途径生成的部分草酸和苏糖酸还可以在草酸氧化酶和草酰CoA合酶的作用下变为CO_2[42]（图4）。

3 代谢途径相关基因的表达对AsA水平的调控

在AsA代谢途径中，生物合成和循环再生相关基因对植物体内抗坏血酸积累的调控研究的较多。很多研究分析了不同植物在果实/叶片发育过程或不同外界条件下AsA代谢相关基因的表达对AsA积累的影响，虽然研究结果并不完全相同，但大多数研究认为GME、GGP和GPP是植物体内抗坏血酸积累的关键调控位点，因此这里只对以上三个基因可能的调控机制展开讨论。

研究发现，在猕猴桃花后4-7周，GME和GGP在不同种间表达量差异很大，AsA含量越高的种，以上两个基因的表达越高[43]。另一篇研究中也发现高AsA含量的毛花猕猴桃中GGP的表达量显著高于低AsA含量的山梨猕猴桃中相应基因的表达[44]。拟南芥稳定表达和烟草瞬时表达的结果也表明GME和GGP协同控制AsA含量，其他基因的表达不影响AsA含量[45]。Li等[34]研究了猕猴桃果实发育过程中相关基因的表达与AsA含量的相关性，结果发现GPP是关键的调控位点。枣果实发育过程及不同基因型中AsA代谢的研究也发现GMP和GME在调控AsA代谢过程中发挥主要作用[46]。

另一方面，很有研究发现光照、非生物胁迫和外源生长调节剂也通过影响GGP等基因的表达来调控AsA含量。如Li等[47]发现不同的光照和非生物胁迫可以影响猕猴桃GGP基因的表达，进而调控AsA水平。Gao等[48]和Laing等[49]也分别发现在不同光照度条件下，拟南芥叶片中AsA浓度与GGP基因的表达相关。研究还发现拟南芥GGP有明显的日变化模式，即在早晨表达较高，随后降低，与AsA含量变化趋势一致[50,51]。苹果的遗传图谱也发现AsA含量与GGP基因连锁[12]。Wolucka等[52]还发现当拟南芥和烟草悬浮细胞在受到茉莉酸甲酯处理后，会通过刺激GME的表达提高细胞内AsA含量。

除了在基因转录水平发现GGP和GME是植物体内抗坏血酸积累的重要调控位点，基

因翻译水平的研究也表明了相同的观点。最近 Laing 等[53]在拟南芥中发现了一个 AsA 反馈调控机制。他们发现拟南芥 GGP 基因 5`端非编码区存在一段顺式作用序列，这段序列有一个非经典的起始密码子 ACG，编码一段 60-65aa 的多肽。这段氨基酸能够在高 AsA 浓度时抑制 GGP 的翻译，而在低 AsA 浓度时这段序列不表达，从而植物可以启动 GGP 的正常翻译。这一模式证明 AsA 含量与 GGP 蛋白含量之间具有直接的联系，植物可以通过对 GGP 基因的翻译来反馈调控 AsA 含量。

4 其他基因的表达对 AsA 水平的调控

除了 AsA 代谢相关基因，其他几个基因也能调控植物中 AsA 的积累。根据调控机制可以分为两类：一是通过影响植物 AsA 代谢酶的绝对数量或者活性进行调控的功能基因。如 Wang 等[54]发现光敏形态发生因子 COP9 signalosome subunit 5B（CSN5B）能与 VTC1（GMP）的 N 端互作，这种互作启动了 COP9 signalosome 复合物对 VTC1 蛋白的泛素化降解，从而影响 AsA 的积累。AMR1（for ascorbic acid mannose pathway regulator 1）基因编码的蛋白序列 N 端和 C 端分别有 F-box 和 DUF295 功能域。F-box 功能域与 ORE9 和 UFO 蛋白等有很高的同源性，而这些蛋白均属于 SCF（Skp1-Cullin-F-box）类 E3 泛素连接酶复合体组分，可以通过 SCF 复合物参与泛素化，调控下游靶基因的表达[55,56]。Zhang 等[57]在研究也证实了 AMR1 在拟南芥中能够抑制 L-半乳糖合成途径中 6 个基因的转录，负调控着 AsA 的合成。另有研究发现 VTC3 的氨基酸序列 N 端和 C 端分别编码蛋白激酶和 2C 型蛋白磷酸酶，它的突变体的 AsA 含量变低，但它的作用机制还不清楚，推测可能是通过转录后调控影响 AsA 代谢酶的绝对数量[58]。Sawake 等[59]发现 KONJAC1 和 2 蛋白能够通过提高 GME 的活性来增加植物体内 AsA 的积累。Cho 等[60]也发现钙调蛋白类似物 CML10 能够通过提高 PMM 的活性来增加 AsA 含量。

二是一些转录因子。通过过表达拟南芥 AtERF98 基因及其缺失突变体的研究发现，AtERF98 能够增加 L 半乳糖途径和肌醇途径相关基因的表达，从而提高 AsA 含量。进一步的深入研究还发现，AtERF98 能够结合于 GMP 基因的启动子区而调控其表达[61]。Hu 等[62]也发现番茄 HD-Zip I 型转录因子 SlHZ24 能够通过调控 L-半乳糖途径相关基因的表达来增加 AsA 含量，而且也发现该转录因子能够绑定于 GMP 基因的启动子。

5 通过转基因对 AsA 进行调控

通过生物技术手段对植物体内抗坏血酸进行调控，主要表现为利用转基因技术提高植物体内 AsA 含量。目前这一方面已经取得了很大进展，获得了很多 AsA 含量发生变化的转基因植物（表 1）。

根据转入基因的功能可以分为以下几类：一是转入植物 AsA 生物合成基因，包括涉及 L-半乳糖途径和其他几条可选途径的相关基因。如 Bulley 等[10]将一个强组成型启动子（35S）驱动下的 GGP 基因分别转入番茄和草莓中，结果发现转基因番茄和草莓中 AsA 含量分别比它们的野生型高 6 倍和 2 倍。GME 基因转入拟南芥[63,64,65]和水稻[66]，也使得转基因植物的 AsA 含量提高 1.1-1.6 倍和 1.4 倍。GMP[67]、GPP[63]、GalDH 和 GalLDH[63,68]基因分别转入拟南芥、番茄和烟草等植物后，也能提高转基因植物中 AsA 含量。

除了 L-半乳糖途径的基因，转入其他合成途径相关基因同样可以提高 AsA 含量。如转入草莓半乳糖醛酸途径中的 GalUR 基因的番茄，获得的转基因植株中 AsA 含量分别提升 1.4-2.5 倍[69,70,71]。将肌醇途径的 MIOX 基因转入拟南芥[72]和番茄[67]中，AsA 含量分别提高了 1.5 和 1.3 倍。还有几个研究将酵母和小鼠的 ALO 基因转入植物也获得成功，不同程度地提高了 AsA 含量[73,74]。

二是转入植物 AsA 再生循环基因。转入 DHAR 基因的拟南芥和番茄，其 AsA 含量增加

了 1.3-1.9 倍[64,75,76,77]。转化了 MDHAR 基因的烟草的 AsA 含量也增加了近 2 倍[78]。

三是转入一些调控因子。如 ERF[61]、KONJAC [59]HD-Zip[62]和 Dorf[79]等，也能改变转基因植株中 AsA 的含量。

6 总结和展望

综上所述，AsA在植物生长发育过程中的作用不仅涵盖碳代谢、细胞分裂和生长和植物开花调控等生理功能，还与植物的逆境胁迫响应有关。植物中AsA生物代谢的途径现在已经比较清楚，特别是主要的合成途径——L-半乳糖途径。伴随着对植物AsA代谢途径的深入认识，AsA代谢的调控机制目前成为AsA研究的一大热点。本文综述了植物（园艺植物）AsA生物合成、循环再生、运输和降解等代谢途径，并重点详述了目前发现的转录水平和翻译水平的各种植物AsA代谢调控机制。

虽然目前针对植物AsA代谢调控机制已经有了较深入的进展，但仍有很多方面值得我们继续研究：

（1）植物各个器官均可以生成AsA，但含量差异显著。因此器官特异的表达调控机制是否存在有待研究。

（2）AsA含量可以通过反馈调控机制影响AsA的代谢，那么其他的抗氧化物质是否也可以调控AsA水平？

（3）不同植物种类间AsA水平相差上百倍，表明AsA代谢及其调控机制在各种植物进化过程中经历了不同的选择，那么具体的机制是什么？

（4）目前大多数关于AsA代谢的研究结果均出自模式植物（拟南芥、番茄和烟草等）的研究，包括园艺植物（人类主要的AsA摄取来源）在内的其他植物的研究还很浅显。下一步应该充分挖掘这些植物的种质资源，通过一些特殊的种质材料来深入研究AsA的代谢调控机制，如猕猴桃属不同种间AsA水平差异显著，就是很好的研究材料。

表 1 近年来一些通过转基因技术调控植物体内 AsA 含量的研究

Table 1 Examples of transgenic approaches to regulate ascorbic acid in plants in recent years

转入基因 Gene transformed	基因供体 Gene donor	受体植物 Species transformed	参考文献 References
GMP	拟南芥 *Arabidopsis thaliana*	拟南芥 *Arabidopsis thaliana*	[59]
GMP	拟南芥 *Arabidopsis thaliana*	拟南芥 *Arabidopsis thaliana*	[63]
GMP	番茄 *Solanum lycopersicum*	番茄 *Solanum lycopersicum*	[67]
GME	刺梨 *Rosa roxburghii*	拟南芥 *Arabidopsis thaliana*	[64]
GME	苜蓿 *Medicago sativa*	拟南芥 *Arabidopsis thaliana*	[65]
GME	拟南芥 *Arabidopsis thaliana*	拟南芥 *Arabidopsis thaliana*	[63]
GME	水稻 *Oryza sativa*	水稻 *Oryza sativa*	[66]
GGP	中华猕猴桃 *Actinidia chinensis*	番茄 *Solanum lycopersicum*	[10]
GGP	马铃薯 *Solanum tuberosum*	马铃薯 *Solanum tuberosum*	[10]
GGP	拟南芥 *Arabidopsis thaliana*	拟南芥 *Arabidopsis thaliana*	[63]
GGP	水稻 *Oryza sativa*	水稻 *Oryza sativa*	[66]
GGP	中华猕猴桃 *Actinidia chinensis*	草莓 *Fragaria ananassa*	[10]
GPP	拟南芥 *Arabidopsis thaliana*	拟南芥 *Arabidopsis thaliana*	[63]
GalDH	拟南芥 *Arabidopsis thaliana*	拟南芥 *Arabidopsis thaliana*	[63]
GalLDH	生菜 *Lactuca sativa*	生菜 *Lactuca sativa*	[68]
GalLDH	拟南芥 *Arabidopsis thaliana*	拟南芥 *Arabidopsis thaliana*	[63]
GalLDH	刺梨 *Rosa roxburghii*	烟草 *Nicotiana tabacum*	[80]
GMP+GME	桃 *Prunus persica*	烟草 *Nicotiana tabacum*	[81]
GMP+GME	桃 *Prunus persica*	烟草 *Nicotiana tabacum*	[81]
GGP+GPP	拟南芥 *Arabidopsis thaliana*	拟南芥 *Arabidopsis thaliana*	[63]
GGP+GalLDH	拟南芥 *Arabidopsis thaliana*	拟南芥 *Arabidopsis thaliana*	[63]
GalUR	草莓 *Fragaria ananassa*	番茄 *Solanum lycopersicum*	[71]
GalUR	草莓 *Fragaria ananassa*	番茄 *Solanum lycopersicum*	[79]

GalUR	草莓 *Fragaria ananassa*	番茄 *Solanum lycopersicum*	[69]
MIOX	拟南芥 *Arabidopsis thaliana*	拟南芥 *Arabidopsis thaliana*	[72]
MIOX	番茄 *Solanum lycopersicum*	番茄 Solanum lycopersicum	[67]
ALO	酵母 Yeast	柱花草 Stylosanthes guianensis	[74]
ALO	小鼠 Rat	拟南芥 *Arabidopsis thaliana*	[72]
MDHAR	金虎尾 *Malpighia coccigera*	烟草 *Nicotiana tabacum*	[78]
DHAR	马铃薯 *Solanum tuberosum*	番茄 *Solanum lycopersicum*	[75]
DHAR	新疆梨 *Pyrus sinkiangensis*	番茄 *Solanum lycopersicum*	[76]
DHAR	刺梨 *Rosa roxburghii*	拟南芥 *Arabidopsis thaliana*	[64]
DHAR	中华猕猴桃 *Actinidia chinensis*	拟南芥 *Arabidopsis thaliana*	[77]
ERF98	拟南芥 *Arabidopsis thaliana*	拟南芥 *Arabidopsis thaliana*	[61]
HZ24	番茄 *Solanum lycopersicum*	番茄 *Solanum lycopersicum*	[62]
KONJAC	拟南芥 *Arabidopsis thaliana*	拟南芥 *Arabidopsis thaliana*	[59]
CSN5B	拟南芥 *Arabidopsis thaliana*	拟南芥 *Arabidopsis thaliana*	[54]
Dof22	番茄 *Solanum lycopersicum*	番茄 *Solanum lycopersicum*	[79]

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The Metabolism and Regulation of Ascorbic Acid A Case Study via Model and Horticultural Plant

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Abstract Ascorbic acid (AsA) plays important roles in the growing development and stress resistance in plant. We review the pathways of AsA metabolism, and focus on regulation mechanism of various genes on AsA metabolism by gene transcription, translation and transformation in model and horticultural plant. The reviews may provide insight for future study on regulation mechanism of AsA metabolism in plants, focusing specifically on the horticultural plant.

Key words Ascorbic acid Metabolism Regulation Model plant Horticultural plant